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THE INFLUENCE OF SOME FUNGI ON MALT QUALITY

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DESCRIPTORS

Aspergillus; Barley analysis result; Contamination; Fungi; Gushing; Malt quality.

SUMMARY

Gushing beer has occurred sporadically at one of Pripps' breweries during the last decades. The mycoflora on barley and malt was investigated in order to find the cause of this problem. It was found that malt causing gushing beer was heavily contaminated with the genera *Aspergillus*, *Penicillium* and *Rhizopus*.

The species *Aspergillus fumigatus* and *Aspergillus amstelodami* which appeared on the barley during germination in the malting plant were found to cause the gushing. Evidence was furthermore presented indicating that the conditions in the malting plant might promote the growth of these fungi.

The present investigation was undertaken to evaluate whether fungi on barley also gave rise to other qualitative changes to malt and wort.

Spores and mycelium of *A. fumigatus*, *A. amstelodami* and *Rhizopus oryzae* were therefore added to the second steeping water, the barley was germinated at 14°C and the malt was kilned during 46 hours. Analysis of the malt and wort samples showed that the above-mentioned fungi species were able to raise the extract yield, the amount of soluble nitrogen, the α-amino nitrogen and the attenuation limit, to reduce the extract difference and viscosity, and to alter the colour of the wort samples.

ÜBER DEN EINFLUSS EINIGER FUNGI AUF DIE QUALITÄT DES MALZES

DESKRIPTOREN

Aspergils; Gerstenanalysen (Ergebnis von); Kontamination; Fungi; Wildwerden (Bier); Malzqualität.

ZUSAMMENFASSUNG

Im Verlauf der letzten Jahrzehnte ist gelegentlich überschäumendes (wildgewordenes, gushing Bier) in einer der Braustätten von Pripps aufgetreten. Es wurde die Mykoflora auf Gerste und Malz untersucht, um die Ursache dieses Problems zu finden. Dabei stellte sich heraus, dass Malz, das überschäumendes Bier hervorrief, stark mit den Gattungen *Aspergillus*, *Penicillium* und *Rhizopus* infiziert war.

Die Arten *Aspergillus fumigatus* und *Aspergillus amstelodami*, die während der Keimung in der Mälzerei auf der Gerste auftraten, konnten als Verursacher des Wildwerdens nachgewiesen werden. Weiterhin liegen Beweise dafür vor, das die Bedingungen in der Mälzerei das Wachstum dieser Schimmelpilze begünstigen können.

Die vorliegende Untersuchung wurde angestellt, um zu beurteilen, ob Fungi auf Gerste auch Anlass zu anderen qualitativen Änderungen bei Malz und Würze sind.

Es wurden deswegen Sporen und Mycel von *Aspergillus fumigatus*, *Aspergillus amstelodami* und *Rhizopus oryzae* dem zweiten Weichwasser zugesetzt, die Gerste bei 14°C gekeimt und dann das Malz 46 Stunden lang gedarrt. Die Analysen der Proben von Malz und Würze ergeben, dass die genannten schimmelpilzarten in der Lage waren, die Extraktausbeute, die Menge an löslichem Stickstoff, den α-Aminostickstoff und den Endvergärungsgrad zu erhöhen, die extraktdifferenz und die Viskosität zu erniedrigen und die Farbe der Würzeproben zu ändern.

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AU SUJET DE L'INFLUENCE DE QUELQUES FUNGI SUR LA QUALITE DE MALT

DESCRIPTEURS

Aspergillus; Analyse d'orge (résultat); Contamination; Fungi; Giclage; Qualité du malt.

RESUME

Au cours de ces dernières décennies il y a eu, sporadiquement, de la bière sauvage dans une des brasseries de Pripps. La mycoflore sur l'orge et le malt a été examinée afin de trouver la cause de ce problème. On a constaté que le malt qui causait de la bière sauvage était gravement contaminée par les genres *Aspergillus, Penicillium* et *Rhizopus*.

On a trouvé que c'étaient les espèces *Aspergillus fumigatus* et *Aspergillus amstelodami*, se présentant sur l'orge pendant la germination dans les installations de maltage, qui étaient la cause du giclage. En outre, il a été démontré que les conditions dans les installations de maltage pourraient favoriser la croissance de ces fungi.

Le présent examen a été entrepris pour voir si des fungi sur l'orge étaient également la cause d'autres changements qualitatifs du malt et du moût.

A cet effet on a ajouté des spores et du mycélium de *A. fumigatus, A. amstelodami* et *Rhizopus* à la deuxième eau de trempage, l'orge était germinée à 14°C et le malte était touraille pendant 46 heures. L'analyse des échantillons de malt et de moût a démontré que les espèces de fungi susmentionnées étaient capables d'augmenter le rendement en extrait, la quantité d'azote soluble, l'azote α-aminé et l'atténuation limite, de réduite la différence d'extrait et la viscosité, et de changer la coloration des échantillons de moût.

INTRODUCTION

As reported earlier, we have studied the occurrence of fungal microorganisms on malt in order to discover the cause of the gushing problems experienced by one of our breweries on several occasions during the past few years (1, 2). On the last of these occasions, we were able to confirm that *Aspergillus fumigatus* and *Aspergillus amstelodami* were the root of the trouble. We also found, when we studied malt originating from 4 different European countries, that the fungal microflora on malt was completely different from that on barley. The dominating groups on malt were so-called "storage fungi" such as: *Aspergillus, Penicillium, Mucor* and *Rhizopus* groups.

Strains appearing on nearly all malt, regardless of the land of origin, were *Aspergillus fumigatus* and *Aspergillus amstelodami*. However, field fungus strains which normally occur on healthy cereals were found to only a very small extent in malt. The probable explanation for this is that very few types of field fungus can survive the high temperatures of kilning. Also, several fungus groups are parasites requiring a living cereal germ host for their own existence. As a rule, the malt germs have lost their ability to grow sprouts and are therefore unable to serve as hosts for true parasites.

Another finding that emerged from our previous study was that the gushing was not caused by the use of fungus-contaminated barley in the production of malt. In actual fact, gushing occurred because *A. fumigatus* and *A. amstelodami* were able to grow during the germination period. The conditions favouring the growth of these microorganisms were specifically located to the parts of the barley layer that were particularly deep and where the temperature rose from 14°C to more than 20°C because the capacity of the cooling system was not sufficient to penetrate through these deeper sections of the barley layer.

At the present time, we do not know why fungus on barley and malt causes gushing. Information in this field is lacking although the literature does cite many examples of fungi that exude enzymes, hormones and toxins which can cause changes in the chemical composition of the germinating barley. But, in general, there is very little that has been published which deals with the metabolic interplay between barley in the process of germination and the concurrent presence of microorganisms.

The object of the present investigation has been to clarify whether *A. fumigatus* and *A. amstelodami* — which have been found to cause gushing — give rise to changes in malt and wort which, in turn, lead to other qualitative changes in the beer. We also included studies of *Rhizopus oryzae* in this work because, although this fungus does not actually cause gushing, it has at times been rather prevalent in malt coming from the Swedish malting plant that has given gushing beer.

EXPERIMENTAL

The cultivation of the three fungi was performed in tissue culture flasks on Peptone, Yeast extract, and Dextrose medium. After incubation for 3 weeks at 20°C, the spores were homogenized. A portion of 225 ml of the homogenate was added to the steeping water as described later.

Malting

6 kg of barley was steeped in water at 15°C for 17 h, drained, air-rested for 7 h, and steeped for a further 17 h at 15°C. Germination proceeded for 6 days and the temperature was kept at 14°C, as indicated in the following. The green malt was dried to 4% moisture content by blowing air through the bed for 24 h at 50°C and, subsequently, 22 h at 80°C.

BREWING

15 litres of water at 51°C were added to 4 kg of malt. The pH was adjusted to 5.4 with H_2SO_4. After 45 min, the temperature was raised to 67°C and kept at this level for 40 min. The temperature was then increased to 78°C for 5 min. Lautering and sparging were performed at 78°C and the pH was adjusted to 5.27. After the addition of hops, the worts were boiled for 90 min and clarified. The specific gravity of the wort was adjusted to 1.042.

Fermentation

The wort was aerated to give 75-90% saturation and pitched with centrifuged yeast (1 g/litre). Fermentation was carried out in closed fermentors at 11°C.

Measuring of gushing: After fermentation, storage for 2 weeks, filtration and carbonation, the beer samples were bottled and stored for another 2 weeks. The samples were placed in a rocking box and rocked for 48 h at 10°C. The samples were then taken out, left to rest for 30 min at 20°C, and submitted to 5 inversions. After being allowed to rest for 5 min, the bottles were opened and the amount of beer gushing from each 330 ml bottle was measured in gram. Each figure given for the amount of gushing in the Tables represents the mean of at least three replicate bottles.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of *A. fumigatus*, *A. amstelodami* and *R. oryzae* on malt when the barley is infected with the steeping water. The temperature during germination was kept at 14°C. It can be seen that there is a tendency for increased extract obtained from the malts that were infected with fungi.

	Congress mash			
Moisture content, %	3.5	3.6	3.8	3.7
Extract content, fine grind, % on dry matter	78.5	81.2	79.0	81.4
Extract difference, %	4.6	4.3	3.4	3.2
Saccharification time, min.	10-15	10-15	10-15	5-10
Wort colour, EBC units	3.1	3.5	4.6	4.5
pH	6.08	6.06	5.95	5.82
Soluble N content, mg/l	666	685	763	824
Protein content, %	12.6	12.6	12.6	12.6
FAN, mg/l	152	160	199	210
Viscosity, cp	1.90	1.90	1.80	1.62
Apparent attenuation limit, %	73	74	76	74
Treatment	-	A.F.	A.A.	R.O.

Fig. 1. The influence of *Aspergillus Fumigatus*, *A. Amstelodami* and *Rhizopus Oryzae* on malt quality.

Also, the malt obtained after contaminating the barley with the three microorganisms showed a more modified malt, as illustrated by the smaller difference. Furthermore, the colour of the wort was intensified, and the content of soluble nitrogen and α-amino-nitrogen in the malt increased while the viscosity decreased. It is obvious, therefore, that the three fungi possess proteolytic, amylolytic and other carbohydrate-splitting enzymes which give noticable effects under the conditions prevailing during the malting process.

Having acquired this information about the effects of the three fungi on germinating barley, we decided that we would also see whether beer was

affected by fungal-contaminated steeping water. Tests were carried out with *A. fumigatus* and *R. oryzae* on pilot-plant scale. The analysis of the Congress-mashed malt is given in Fig. 2. The extract content of the malt increased, slightly as did the attenuation limit, soluble nitrogen, α-amino-nitrogen and colour. The *R. oryzae* microorganisms, in particular, contribute towards the

	CONGRESS MASH			
Moisture content, %	3.8	5.2	4.7	4.5
Extract content, fine grind, % on dry matter	78.1	79.1	80.2	79.9
Extract difference, %	3.8	1.5	0.9	1.6
Saccharification time, min.	10-15	10-15	10-15	10-15
Wort colour, EBC units	3.0	3.8	4.5	3.8
pH	6.1	6.0	6.0	5.9
Soluble N content, mg/l	645	695	774	862
Protein content, %	12.2	12.4	12.4	12.2
FAN, mg/l	141	145	185	225
Viscosity, Cp.	1.82	1.49	1.52	1.50
Apparent attenuation limit, %	76.2	79.9	80.0	78.8
Treatment	–	A.F.	A.F.	R.O.
Contaminated kernels, %		32	69	70

Fg. 2. The influence of *Aspergillus Fumigatus* and *Rhizopus Oryzae* on malt quality.

heavy increase in α-amino-nitrogen and soluble nitrogen. On the other hand, there is a reduction in the extract differences and in the viscosity. The pilot plant wort analyses obtained for these malts are given in Fig. 3. As in the case of the first set of experiments, the results thus showed an apparent increase for the attenuation limit, for the colour intensity, for the α-amino-nitrogen and for the soluble nitrogen, whereas there was a considerable decrease in the viscosity and the extract-difference. Fig. 4 shows that all these changes in the wort persist in the resultant beer. It can be seen from the Figure that the attenuation limit for the beer increases, which means a higher alcohol content in the beers brewed with malt that had been contaminated with the two fungal strains. It can also be seen that all the beers were completely attenuated, which means that the excess of extract could be utilized by the yeast.

	Congress mash			
Original gravity, %P	10.15	10.49	10.64	10.44
Apparent attenuation limit, %	70.3	74.9	76.9	80.6
Colour, EBC units	6.5	7.75	8.8	10.5
Viscosity, cp.	2.23	1.76	1.66	1.62
pH	5.19	5.04	5.18	5.24
FAN, mg/l	143	199	239	268
Soluble N cont., mg/l	770	924	1069	1092
Treatment	-	A.F.	A.F.	R.O.
Contaminated kernels, %		32	69	70

Fig. 3. The influence of *Aspergillus Fumigatus* and *Rhizopus Oryzae* on pilot plant wort composition.

The Figure also shows that both fungi cause a strong intensification of the colour of the beer. The change in colour is probably caused by the increased content of reducing sugars and free amino acids. If we compare Fig. 2 and Fig. 4, it can be seen that the colour of the malt does not correspond to the colour of the beer. Very likely, this is because mashing according to the Congress method does not lead to the maximum colour response for the Maillard reaction, as has been discussed by Stage and Scriban (3, 4).

Fig. 4 also gives the information that the fungal-contaminated beer contains an abnormally high residue of free amino acids. In comparison to the reference beer, there is 2-3 times as much free amino acid in the contaminated product. The Figure also gives the data for the gushing properties of the beer. When 32% of the malt grains are infected with *A. fumigatus*, about 89 gm gushes out of the 330 ml-bottles. With grain infected to 69%, the quantity running over amounts to 161 gm. On the other hand, *R. oryzae*-infected grain does not give rise to any gushing although this organism has been found to affect other properties, as I have already described. A study of the foam-stability of these beers using the Klopper method (5) shows that beers brewed from fungal-contaminated malt maintain perfectly normal foam-stability in comparison with the reference beer.

We can conclude from the Figures shown so far that the strains of fungi studied possess the ability to increase the content of free amino acids in malt and wort, and that, as a result of the activity of these organisms, the content of free amino acids in the beer is also increased. An interesting question is whether the proportions of amino acids in the wort are changed to such a large

degree that the metabolism of the yeast is affected? Generally speaking,

	Congress mash.			
Original gravity, % P	9.92	10.34	10.56	10.53
Alcohol content, w/w %	3.00	3.43	3.47	3.68
Apparent gravity, % P	2.90	2.55	2.49	1.94
Fermentable residue, % P	0.02	0.00	0.05	0.01
Apparent degree of fermentation, %	70.8	75.8	76.4	81.6
Apparent attenuation limit, %	71.0	75.8	76.9	81.7
Difference	0.2	0.0	0.5	0.1
Colour, EBC units	4.3	6.0	6.9	7.4
Bitterness, EBC units	19.0	19.4	20.7	20.4
pH	4.31	4.30	4.49	4.55
FAN, mg/l	50	83	126	145
Soluble N content, mg/l	545	674	787	799
Treatment	-	A.F.	A.F.	R.O.
Contaminated kernels, %		32	69	70
Gushing, gm.	0	89	161	0
Foam stability	normal			

Fig. 4. The influence of *Aspergillus Fumigatus* and *Rhizopus Oryzae* on pilot beer composition.

however, no marked deviations were registered which would be likely to cause changes in the aroma of the beer.

Our earlier studies showed that malt, in common with insufficiently dried or damaged cereals, is contaminated by several different families of storage fungi which might alter the chemical composition of the grain germs if conditions are such that the microorganisms in question are allowed to grow. The information resulting from the present study illustrates that the chemical composition of malt and wort *can* undergo changes under the conditions prevailing in a malting plant. Another interesting question, is whether the aroma of the beer can also be affected by the presence of fungi in the malt? We studied this problem in a triangle test, using beer that had been brewed with malts infected with *A. fumigatus* and, respectively, *R. oryzae*, and with beer brewed from uninfected malt as reference product. In these

taste-tests, 11 tasters out of the 14-man panel were able to identify the beer that had been brewed with malt contaminated with *A. fumigatus* in the steep water. 13 members of the panel identified beer brewed with malt contaminated with *R. oryzae*. The *A. fumigatus* beer was characterized as having a pronounced roughness and a staling flavour. According to the panel, there were no special off-flavour in the *R. oryzae* beer.

Our studies have thus shown that malt, wort and beer can all be affected by the fungi named here. In our experiments, the germinating barley was infected with contaminated steep water, as already described. Although the pilot-plant tests were run under conditions that conformed as closely as possible to the conditions in the malting plant from which the gushing problem originated, it is quite clear that contamination which is arranged by submitting the barley grains to spore-infected steeping water is a process which differs very much indeed from natural contamination. The questionability of the situation stems from a possible exaggeration of the effects of the fungi, for instance in the case of the malt kernals being exposed to an exceptionally large number of spores in the steep water, as a result of which the extent of the effects may be misleading. On the other hand, the actual number of spores is probably of less importance than other factors since the spores themselves are metabolically inactive. It is instead the conditions prevailing during the germination period which are decisive-conditions which permit the spores to grow and develop mycelium with a high degree of activity that can affect the composition of the barley kernal.

In order to elucidate whether the conclusions we have drawn from the trials in our pilot-plant are also applicable to the situation in the malting plant, we have compiled the analytical data obtained over a period of time during which one of the malting plant produced malt which caused gushing beer. Fig. 5 shows the occurence of fungi during this period. For comparison, the Figure also gives the content of fungi in the malt for the period following the gushing, when the problem had disappeared. It can be seen that *A. fumigatus* and *R. oryzae* are both present in large quantities during the gushing period. Afer the germination floors, conveyor, belts, the kiln and so forth at the malting plant had been subjected to cleaning measures, the content of fungi in the malt was reduced and there was no more gushing. The malt analyses from the gushing period are given in Fig. 6. It will be seen that the extract difference and the viscosity were lower during the gushing period, and the colour intensity and α-amino nitrogen higher, compared with the subsequent period. It must be pointed out that the barley used during the period in question was practically free from the strains of fungi given in Fig. 5.

Therefore, the use of fungal-contaminated barley cannot be given the blame for the gushing which arose. The gushing phenomenon was instead caused by conditions prevailing in the malting plant during the germination period which permitted and favoured the growth of the *A. fumigatus* microorganisms.

In other words, the experiences obtained from the malting plant show the same tendency as the pilot-plant experiments in which barley used had been infected with spore-contaminated steep water. We therefore maintain that situations *can* arise in the malting plant which result in conditions favouring the growth of fungi and which, in turn, alters the chemical composition of the

Malt samples No.	1	2	3	4	5	6	7	8	9	10	11
Aspergillus spp.	0	0	0	0	0	2	2	46	9	14	7
A. fumigatus	85	100	80	76	74	1	1	0	1	1	3
A. amstelodami	16	0	12	32	28	5	40	26	3	0	4
A. flavus	0	0	1	1	0	0	1	0	1	0	0
Mucor spp.	0	0	0	0	0	26	9	4	51	1	47
Penicillium spp.	12	0	14	16	18	18	11	26	8	6	44
Rhizopus spp.	90	50	75	88	37	8	21	5	2	1	5
	Period of gushing beer.					No gushing.					

Fig. 5. Storage fungi on malt. Contaminated kernels as percentage of total number.

Moisture content, %	3.6	3.8
Extract content, fine grind, % on dry matter	80.2	80.4
Extract difference, %	1.13	1.77
Colour, EBC units	4.06	2.94
Viscosity, Cp	1.54	1.71
FAN, mg/l	204	180
Gushing	+	0

Fig. 6. The influence of fungi on malt quality.

malt and leads to the subsequent qualitative changes in the beer.

According to our experience, the growth of fungi takes place particularly during the germination of the barley under conditions which are optimal for fungal growth with respect to temperature, moisture and the availability of nutrients from the barley.

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DISCUSSION

L. Munck (Denmark): Comment: In 1976 I stayed in Hyderabad India where I bought 12 different beer bottles of 12 different brands of which 9 showed a typical gushing. This points to the problem of obtaining a microbiologically sound malting in the tropical countries where the barley could be infected either in the malthouse or during storage in the wet monsune season. At harvest in India the barley should be sound because it is harvested during the dry season.

A.D. Rudin (G.B.): Q: Was the viscosity of the gushing beer different from that of the normal beer?

A: Yes.

H. Dellweg (B.R.D.): Q: These fungi sometimes produce highly toxic compounds. Did you look also to these fungal toxins in malt preparatives and could it eventually happen that toxins appear in concentrations higher than it would be allowed by legislative regulations?

A: Yes, this has been tested with A.fum., as this fungi has been found on many malt samples. Barley was thus infected with the steeping waters as described in the paper. 100% of the kernels were contaminated in this way. The beer produced was freese-dried and was mixed with the feed of a couple of rats during 2 months. During the test-period these animals consumed at about 500 times more beer than a human being expressed per kg body weight. After the test period the animals were decapitated and their intestines examined histopathologically. The conclusion was that no toxic substance had been transfered to the beer.

M.A. Amaha (I): Comment: I'd like to congratulate you for your excellent work on the relationship between the mould contamination and the gushing in beer. Since 1973 when we presented our first paper on gushing in the E.B.C. Congress, Salzburg, we are still continuing our study mainly in the direction of isolating and identifying the gushing factors produced by moulds. We have recently succeeded in isolating a new factor from a *Penicillium* strain that is morphologically identified as *Pen chrysogenum* (a famous Penicillin-producing species). This newly isolated factor is extremely active in inducing gushing; it induces gushing when added to normal beer at a very low concentration as low as 0.02 ppm. The other authentic strains of *Pen. chrysogenum* and related species were also found to produce a gushing factor of similar nature, i.e. peptide. We think, therefore, that it is essential to identify the mould isolates to the level of species, as you have done in your study.

Q: Now, I wonder if you have done some works towards isolation and identification of the gushing factors produced by your *Aspergillus* strains. I am very much interested in knowing what is the chemical nature of gushing factor of your strains.

A: No work has been done in this area.

T. Wainwright (G.B.): Q: Have you made mixtures of infected and uninfected malts (to give for example 34% infection) and observed the amount of gushing? Have you looked to see if the differences observed in the wort and beer are due to enzymic attack during mashing rather than during malting? For instance have you made extracts with cold water?

A: No work has been done in this direction.

M. Jones (G.B.): Q: Have you any indication of the level of mould infection necessary to produce gushing?

A: Yes. In the case of A. fumigatus 30-40% of contaminated kernels is enough to produce gushing at the experimental conditions we have used.

A.D. Portno (G.B.): Q: Is an interaction between the fungus and the germinating barley essential for gushing to occur? Can gushing be induced by adding a culture of the organism grown in pure culture to the beer or to the wort prior to boiling?

A: We think it is a question of interaction between the fungus and the germinating barley.